"Certain Laws of Variation. I. The Reaction of Developing Organisms to Environment." By H. M. VERNON, M.A., M.D., Fellow of Magdalen College, Oxford. Communicated by Professor E. RAY LANKESTER, F.R.S. Received March 7,— Read March 29, 1900.

In a former paper[*] it was shown that the ova of the Echinoid *Strongylocentrotus lividus* were extraordinarily sensitive to their environmental conditions at the time of impregnation. For instance, by keeping the mixed ova and spermatozoa in water at about 26° or 8° C. for an hour, the plutei obtained after eight days' development were some 5 per cent. smaller than those from ova kept at about 20° at the time of impregnation. It was even found that the effect produced was nearly as great if the time of subjection to the abnormal temperature were reduced to one or three minutes, though if reduced to ten seconds it was not so great. This latter result was probably due to the time being insufficient for all the ova to become impregnated at the abnormal temperature.

These observations have now been repeated and confirmed, and in addition others have been made upon the reaction of the ova to environment in the later stages of their development. It has thereby been found that the degree of this reaction diminishes in more or less regular proportion from the time of impregnation onwards.

The method of experiment is fully described in the above-mentioned paper, so it will be sufficient to state here that it consists in shaking pieces of the ovaries and of the testes of several specimens of the Echinoid in small beakers of water, and then bringing portions of the contents to the required abnormal temperature. These portions are then mixed, and after an hour the temperature is gradually brought to the normal by floating the beakers in large vessels of water. The now impregnated ova are then poured into covered jars holding $2\frac{1}{2}$ to 4 litres of water, and after eight days the plutei into which they develop are killed and preserved, and measured under the microscope with a micrometer eye-piece in groups of fifty. In addition to these plutei, others are obtained in each case from ova impregnated at a normal temperature, but allowed in all other respects to develop under similar conditions. These constitute the normal or standard larvæ, from which the variations in the mean size of the other larvæ are calculated. The particular dimension measured was the length of the calcareous skeleton of the "body" of the larva, that of the arms as a rule not being determined in the present research.

The results obtained, both in the old and the present series of experi-

* 'Phil. Trans.,' B, 1895, p. 577.

ments, are given in the accompanying table, those of the old series being the means of several observations :—

Number of observations.	Time of exposure to abnormal temperature.	Temperature of impregnation of		Percentage diminution in size produced.
		Normal larvæ.	Abnormal larvæ.	
		°	°	
Old series {	8...	1 hour	19·9	8·7	4·2
	5...	,,	19·9	25·5	5·2
	4...	1 or 3 mins.	19·6	8·0	3·3
	3...	,, ,,	19·8	25·5	5·1
	4 ..	10 secs.	19·4	7·7° or 25·7°	1·7
New series {	1...	1 hour	14·2	1·0	3·6
	1...	,,	,,	8·3	4·2
	1...	,,	,,	25·5	9·4
	1...	,,	,,	27·7	6·0

Here it will be seen that, on an average, a more unfavourable effect was produced by exposure of the ova to a temperature of about 25·5° for an hour or a minute at the time of impregnation, than to one of about 8°. Thus 5·7 per cent. diminution was produced in the 10 observations at 25·5°, and only 3·9 per cent. in the 14 at about 8°.

All these observations were made on the pluteus of *Strongylocentrotus lividus.* A further series was also made with the pluteus of *Sphærechinus granularis.* This was found to be a somewhat less variable and less reactive organism, but even in its case a most distinct effect was produced. The dimension measured in this larva was likewise the body length.* The results obtained were the following :—

Time of exposure to abnormal temperature.	Temperature of impregnation of		Percentage diminution in size produced.
	Normal larvæ.	Abnormal larvæ.	
	°	°	
1 hour	17·4	12·3	7·7
,,	19·5	10·9	1·1
,,	18·8	10·4	4·6
,,	22·8	12·0	2·6
,,	22·1	27·1	2·5
5 minutes	,,	,,	4·3
1 hour	20·9	26·7	0·9
5 minutes	,,	,,	0·9

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* For figures of these larvæ, *vide* 'Phil. Trans.,' B, 1898, p. 468.

Larvæ impregnated for an hour at about 11° were, on an average, 4·0 per cent. smaller than the normal, or practically the same as in the case of *Strongylocentrotus* larvæ. An abnormally high temperature does not seem so effective, however, judging from the few results available. Thus one hour's exposure to about 27° caused only 1·7 per cent. diminution, and five minutes' exposure 2·6 per cent. It should be mentioned that both in this case and that of *Strongylocentrotus*, the conditions of the short-time exposure experiments differed in one respect from the others, as the beakers of abnormally cooled or warmed ova were poured directly into jars of water at normal temperature, and were not first gradually warmed or cooled. That the shock of this sudden change of temperature cannot be held accountable for much of the effect produced, is proved by the fact that in those experiments in which the time of exposure was reduced to ten seconds, only 1·7 per cent. diminution in the size of the larvæ was produced altogether.

Experiments were now made to determine the effect of exposure to abnormal temperatures during later stages of development. In each case all the ova were kept for the first hour during impregnation at the same temperature, and were then divided up into two portions, which were poured into jars of water at different temperatures. In the first experiment, made in March, the temperature of the Aquarium tank water was on an average 12·9°, or distinctly low. Some of the ova, after an hour's impregnation at 13·2°, were accordingly poured into a jar of water kept at 22°. At various later periods the contents of this jar were stirred up, and portions of it poured into smaller jars, which were then transferred to the tank of running water at 12·9°. The temperature of 22°, which previous experiments had shown to be about the most favourable for the development of the larvæ, was maintained practically constant by keeping the jar in a larger vessel of water, which in its turn rested on the top of a water-bath warmed very slightly by means of a gas flame provided with a regulator. The results obtained in this experiment are given in the following table, the body length of the normal larvæ, or those kept at 12·9° during the whole of their development, being taken as 100 :—

Conditions.	Size.	Percentage increase per hour.	During hours
Normal larvæ (12·9°)	100·00		
1—11 hours at 22°	99·96	nil	1—11
1—28 ,, ,, ...	110·86	0·40	1—28
1—71 ,, ,, ...	116·25	0·125	28—71

For some unaccountable reason, the larvæ developing from ova kept

only ten hours at 22° were apparently not affected. As subsequent results will show, this was doubtless due to some error. Those from ova kept from the end of the 1st to the end of the 28th hours were, however, increased nearly 11 per cent., or on an average 0·40 per cent. per hour for each of these hours. The larvæ from ova kept seventy hours at 22° were increased by about 16 per cent., so the additional forty-three hours produced an extra increase of 5·39 per cent. On an average, therefore, the increase during the 29th to 71st hours amounted to only 0·125 per cent. per hour, or less than a third as much as for the earlier period.

In the next experiment, made in April, the temperature of the tank water was 13·8°. In this case we see that by keeping the developing

Conditions.	Size.	Percentage increase per hour.	During hours
Normal larvæ (13·8°)	100·00		
1—8 hours at 23°	107·55	1·08	1—8
1—19 ,, ,, ...	111·60	0·37	8—19
1—43 ,, ,, ...	109·76	0	19—43
1—192 ,, ,, ...	110·88	0	19—192

ova during the 1st to 8th hours at 23°, the larvæ were increased 7·0 per cent. in size, or 1·08 per cent. per hour. For the 1st to 19th hours the increase was 11·6 per cent., or, on an average for the 8th to 19th hours, 0·37 per cent. per hour. Again, therefore, the increase per hour for the later period is only about a third that for the earlier. After the 19th hour, apparently no further increase in size was produced. Probably this is an error, but in any case the effect must have been very slight. It should be pointed out that there is probably in almost every case a possible experimental error of some 2 per cent. in the determination of the growth of these larvæ, and occasionally, as we saw in the preceding experiment, this error may for some unknown reason be considerably greater.

In the next experiment only approximate results can be calculated. Thus larvæ were grown at respectively 13·3° and 20·3° during the whole period of development, but some of them were also preserved and measured after only 3½ days' growth. The following values were obtained :—

	3½ days.	8 days.
Kept at 13·3°	88·28	100·00
,, 20·3°	109·63	111·98

Here we see that the larvæ grown only 3½ days at 20·3° are 9·63 per cent. larger than those grown 8 days at 13·3°. If they had been kept

an additional $4\frac{1}{2}$ days at 13·3° they would doubtless have grown some-what more. The increase for the 1st to 84th hours is therefore some-what more than this 9·63 per cent., though less than 11·98 per cent. Let us take it as 10·80 per cent., or 0·130 per cent. per hour. Again we see that the larvæ kept 8 days at 20·3° are only 2·35 per cent. larger than those kept $3\frac{1}{2}$ days at this temperature. Hence the maximum effect capable of being produced by the more favourable temperature during this 84th to 192nd hour must be somewhat less than 0·022 per cent. per hour, or not a fifth of that produced in the earlier period.

The next experiment was made in July. After an hour's impregna-tion at 22·7°, some of the ova were poured into a jar of water which stood in another jar which was surrounded by water and ice. By this means the developing ova were kept at about 12°. Every few hours the water was stirred up and portions of it poured into jars, which were then transferred to the tank water. This had a mean temperature of 22·5°. The following results were obtained :—

Conditions.	Size.	Percentage diminution per hour.	During hours
Normal larvæ (22·5°).......	100·00		
1—6 hours at 12°..........	93·61	1·28	1—6
1—10 ,, ,, ...	92·37	0·31	6—10
1—21 ,, ,, ...	90·09	0·21	10—21

Here we see that the effect produced during the 1st to 6th hours was four times as great as that during the 6th to 10th hours. In another experiment some ova, which had been impregnated at 1° C., and had thereby given rise to larvæ 3·6 per cent. smaller than the normal, were kept for the next eight hours at 6°. The larvæ resulting therefrom were still 9·34 per cent. smaller, or, on an average, were diminished 1·17 per cent. for each hour of exposure to the abnormal temperature.

As it was found somewhat troublesome to keep a considerable volume of water some twelve or fifteen degrees below that of the atmosphere for many hours, the rest of the observations were made on the effects of keeping the developing ova at a higher temperature than the normal. As the temperature of the air in the summer months at Naples, where these experiments were made, varies but little from day to day, it was easy to keep the water in a tank holding about 30 litres at a practically constant temperature throughout the experiment. In fact, it did not vary more than 0·3° or 0·5° at the most. In such a tank, if left un-covered, the temperature of the water was found to fall by evaporation to about 25°, or about 2° lower than that of the atmospheric tempera-ture. By covering it up, this could be diminished if wished, and small

quantities of hot or cold water could be added to bring the temperature to exactly what was required. The room in which this tank was kept was shut up closely at night so as to prevent cooling.

In the first experiment some of the ova, after an hour's impregnation at 22·2°, were kept for varying periods in this tank of water at 26·0°, and portions of them transferred in smaller jars to Aquarium tank water at a mean temperature of 23·5°. The following results were obtained :—

Conditions.	Size.	Percentage variation per hour.	During hours
Normal larvæ (23·5°)	100·00		
1—4 hours at 26°	88·33	− 3·89	1—4
1—8 „ „	88·90	+ 0·14	4—8
1—12 „ „	94·56	+ 1·42	8—12
1—22 „ „, ...	99·31	+ 0·47	12— 22
1—144 „ „	98·43	− 0·007	22—144

Here we see that three hours' exposure of the developing ova to a temperature of 26° produced a diminution of 11·7 per cent. in the size of the larvæ. Further exposure, on the other hand, not only failed to produce a further diminution of size, but gave an actual increase, which gradually became more and more marked. It is obvious, therefore, that a temperature of 26°, though harmful to the ova in their earlier stages of development, becomes advantageous in the later stages. The reason of this will be made evident further on. It might be thought at first sight that this and other similar experiments in which the environment produces a varying effect, could be of no use in deciding the question under discussion. By judicious selection of certain of the values, however, useful results are obtainable. Thus, in the present instance, we see that by the end of the 8th hour the favourable action of the high temperature has already established itself, and it remains established from that time onwards. All results obtained after this period are, therefore, of value, and the figures which show that the effect produced between the 8th and 12th hours is three times as great as that between the 11th and 22nd hours, are genuine ones. The apparent slight diminution of size occurring between the 22nd and 144th hours is doubt-less due to experimental error. In all the observations made during the summer months the larvæ were killed and preserved after only six days' growth, instead of eight. This was because they practically reach their maximum size in this period, the rate of growth being so much greater than at the lower temperatures experienced in the spring.

In the next experiment, the adverse effect produced during the first few hours' exposure was extraordinarily great, so that the favourable

influence of the later hours was only very partially able to counteract it. Thus, during the 1st to 4th hours, the diminution effected was no less than 6·45 per cent. per hour. There was even a slight additional diminution during the next $3\frac{1}{2}$ hours, but after that the increase in size noticed in the above experiment set in. It is probable that an un- favourable effect persisted even to the first portion of the $7\frac{1}{2}$ to 11 hour

Conditions.	Size.	Percentage variation per hour.	During hours
Normal larvæ (24·2°)	100·00		
1—4 hours at 26·0°	80·64	−6·45	1—4
1—7½ „ „	79·24	−0·40	4—7½
1—11 „ „	80·21	+0·28	7½—11
1—22 „ „	84·25	+0·20	11—22
1—144 „ „	87·27	+0·025	22—144

period, as the percentage increase per hour is considerably less than one would expect. The only values which are unequivocally genuine are, therefore, the last two. From these we see that the effect produced between the 22nd and 44th hours is only an eighth of that between the 11th and 22nd hours.

It was thought that perhaps the very marked diminution produced in the size of the larvæ might be in part due to the rather sudden changes of temperature to which the developing ova were subjected. These changes were not, as a matter of fact, by any means remarkably sudden, as the water in which the ova were placed after impregnation took about fifteen minutes to attain its temperature of 26°, whilst the reverse change from 26° to 24·2°, the temperature of the tank water in this experiment, took about ten minutes. Still, to test this supposition, some of the ova used in this experiment were subjected to several changes of temperature. Thus, one portion, directly after the first hour's impregnation, was kept three hours at 26°, then seven hours at 24·2°, then fourteen hours at 26°, and the remainder of the time at 24·2°. The size of the larvæ obtained therefrom was 85·89, or, if anything, somewhat larger than one would have expected. Another portion of the ova was kept for the 1st to 7½th hours at 24·2°, the 7½th to 22nd hours at 26·0°, and the remainder of the time at 24·2°. The size of these larvæ was 94·93, or 5 per cent. less than the normal. One would have expected them to be if anything slightly larger than the normal, as they were kept at the lower and favourable temperature during the first 6½ hours. Still, these two experiments, taken together, show that the effect of even several changes of temperature can only be slight.

Still, again, it was thought that the vigorous stirring of the water which was necessary in order to distribute the organisms evenly through it previous to withdrawal of a portion, might perhaps exert a retarding influence on development. However, this was evidently not so, as, in two experiments, in which the water was absolutely unstirred throughout, the resulting larvæ varied by respectively + 2·0 and − 2·6 per cent. from those derived from frequently-stirred water.

In the last experiment to be described the developing ova were kept at 25° and not at 26°. Consequently, the diminution produced in the size is not so great. In this case, also, two parallel series of observations were made, one with *Strongylocentrotus* ova as usual, and another with the ova of *Sphærechinus granularis*. In the former case, the unfavourable effect of the high temperature persisted till the end of the

Conditions.	*Strongylocentrotus* larvæ.		During hours	*Sphærechinus* larvæ.	
	Size.	Percentage variation per hour.		Size.	Percentage variation per hour.
Normal larvæ (23·3°)	100 ·00	..	..	100 ·00	
1—4 hours at 25° ..	93 ·95	− 2 ·02	1—4	97 ·56	−0 ·81
1—9 ,, ,,	92 ·64	− 0 ·26	4—9	94· 20	−0· 67
1—21 ,, ,,	97 ·68	+ 0 ·42	9—21	93 ·96	− 0 ·02
1—144 ,, ,,	96 ·88	− 0 ·007	21—144	95 ·17	+ 0 ·009

9th hour, and, in the latter, until at least the 21st hour, and possibly even later. The *Sphærechinus* ova did not react so much to the environment as the *Strongylocentrotus*, just as was found to be the case in the experiments on the effect of temperature at the time of impregnation. Still these observations on *Sphærechinus*, as far as they go, more or less support the conclusion drawn from the *Strongylocentrotus* experiments, viz., that there is a diminishing reaction to environment as the stages of development progress.

We see, then, that in all of these four sets of observations the originally unfavourable influence of the high temperature is later on converted into a favourable one. What is the cause of this? No absolute explanation was arrived at, but some observations made on the maximum or death temperatures of the developing ova gave a very satisfactory partial explanation. In these observations portions of the water containing the ova in various stages of development were placed in a beaker, and this was placed in a larger beaker of water which was gradually warmed. The beaker containing the ova was continuously stirred with a thermometer, and when the required tem-

perature had been reached it was removed from the warm water and quickly cooled down by a stream of cold water. After keeping for twenty-four hours, corrosive sublimate was added to kill off any of the embryos still surviving, and they were all collected in a small glass cell and examined under the microscope. From the different stages of development attained by the developing ova killed at the time of heating, and those only killed twenty-four hours later by the sublimate, one could easily determine the effect of the various degrees of high temperature.

For ova at the time of impregnation the fatal heat temperature is probably about 28·5°. Thus only 31 per cent. of some ova heated to 27·7° at the time of impregnation were found to have developed to normal blastulæ twenty-four hours later, whereas some of the same ova impregnated at a normal temperature (14·2°) were all found without exception to have reached the blastula stage. On the other hand, in another case not a single ovum out of a number heated to 30° at the time of impregnation showed any sign of normal development twenty-four hours later.

As regards subsequent stages, portions of some developing ova, four hours after impregnation, were heated to respectively 29°, 32°, 35°, and 38°. Next day all the embryos heated to 29° and 32° had nearly or quite arrived at the pluteus stage, whilst none of those heated to 35° or 38° had got further than the half-formed blastula stage. The fatal temperature must therefore have been between 32° and 35°, or say 32·5°. Other portions of the same stock of developing ova were heated in a similar manner twelve hours after impregnation. Next day all those heated to 29°, 32°, and 35° had arrived at the full or semi-pluteus stage, whilst all of those heated to 38° were either normal blastulæ or blastulæ just beginning to invaginate. The death temperature in this case must therefore have been about 36·5°. Still other portions of the same stock of embryos were heated to various temperatures twenty-eight hours after impregnation. They had now arrived at the free-swimming pluteus stage, and hence it was quite easy to determine by naked-eye observation what effect had been produced. Of the plutei heated to 37°, none were affected, but all of those heated to 39° sank to the bottom of the beaker in a few minutes. However, about a third of them had recovered an hour after, and all of them had recovered several hours after. None of these plutei were heated above 39°, so the actual death temperature was not determined; but other results showed that the death temperature is only slightly above the heat paralysis temperature, so one may conclude that it was in this case about 39·5°.

On heating some of the six days plutei obtained from the same stock of ova, it was found that a quarter of an hour after heating, three-fourths of those heated to 39° had sunk to the bottom of the beaker,

and all of those heated to 40° and 41°. After an hour, all of those heated to 39°, and half of those heated to 40°, were free-swimming. After four hours four-fifths of those heated to 40° were free-swimming, but none of those heated to 41° had recovered. The death temperature was therefore about 40·3°.

These death temperature observations perhaps become more striking if put in tabular form. Thus :—

Stage of development.	Time after impregnation.	Death temperature.
Strongylocentrotus ova	..	28 ·5°
Semi-blastulæ	4 hours	33 ·5
Blastulæ and semi-gastrulæ	12 ,,	36 ·5
Plutei and semi-plutei	28 ,,	39 ·5
Plutei	6 days	40 ·3

It should be remarked that the embryos used in these observations had been kept at 26° during development. Six days' plutei obtained from the same stock of ova, but allowed to develop at 23·5° instead of 26°, were found to have a death temperature of 39·3°. Thus the higher temperature of development had produced a certain amount of acclimatisation.

The bearing of these results on the curious double effect of exposure of the developing ova to high temperature is obvious. Thus, if a temperature of 29° is fatal to the vitality of ova at the time of impregnation, the temperatures a few degrees below this are doubtless unfavourable to development. Still lower temperatures, on the other hand, are known to exert a favourable influence. Now as in the course of development the death temperature gradually rises, one is quite justified in concluding that the lower limit of the unfavourable temperature rises too, and very probably to a more or less similar extent. In the above experiments it was found that up to the end of four hours a temperature of 26° was distinctly unfavourable to growth. During the next four hours it was more or less neutral, but after this time it was most distinctly favourable. Now the present observations show that between the 4th and 12th hours the death temperature rises about 3°, so what was an unfavourable temperature to the earlier stage of development may have become converted into a favourable one to the later stage.

Let us now return to the results on the effects of temporary subjection to abnormal temperatures. These were obtained under such a variety of conditions that one is scarcely warranted in grouping them all together, but the majority of them can be split up into three more or less homogeneous groups. In one the so-called normal larvæ were

kept at about 20°, and the abnormal ones were kept for varying numbers of hours at about 8°, whereby a negative effect on growth was produced. In another, the normal larvæ were kept at 13°, the abnormal at 22°, a positive effect being produced, and in the third the normal larvæ were kept at about 24° and the abnormal ones for varying periods at about 26°, whereby a negative effect, followed by a positive one, was produced.

Normal larvæ at 20°, abnormal at 8°.			Normal larvæ at 13°, abnormal at 22°.			Normal larvæ at 24°, abnormal at 26°.		
Time of exposure in hours.	Mean time in hours.	Per cent. variation in size per hour.	Time of exposure in hours.	Mean time in hours.	Per cent. variation in size per hour.	Time of exposure in hours.	Mean time in hours.	Per cent. variation in size per hour.
0—1	0·5	−4·14	—	—	--	0—1	0·5	−5·92
1—6	3·5	−1·28	—	—	—	1—4	2·5	−6·45
1—9	5·0	−1·17	1—8	4·5	+1·08	1—4	2·5	−3·89
6—10	8·0	−0·31	1—11	6·0	0·0	1—4	2·5	−2·02
10—21	15·5	−0·21	8—19	13·5	+0·37	8—12	10·0	+1·42
—	—	—	1—28	14·5	+0·40	9—21	15·0	+0·42
—	—	—	19—43	31·0	0·0	11—22	16·5	+0·20
—	—	—	1—84	42·5	+0·130	12—22	17·0	+0·47
—	—	—	28—71	49·5	+0·125	21—144	82·5	0·0
—	—	—	19—192	105·5	0·0	22—144	83·0	+0·025
—	—	—	84—192	138·0	+0·022	22—144	83·0	0·0

The results are arranged in this table according to the times during which the larvæ were exposed to abnormal conditions. The means of these times are also given, as comparisons are thereby rendered easier. In the first line of the left portion of the table is given the average effect produced in the ten experiments already quoted, in which the ova were kept at about 8° at the time of impregnation. (The experiments in which the time of exposure was one to three minutes have been omitted, as the effect produced in this case was probably something special, directly connected with the act of impregnation.) The results in this group of observations show a fairly regular and very rapid diminution in the effect produced on the size of the larvæ with progress in development, but unfortunately they extend only to the 21st hour. The results in the middle portion of the table extend to the 192nd hour, but they are very irregular. Nevertheless they also, on the whole, show a rapidly diminishing effect. The results in the right portion of the table bear out this result more fully. In the first line is given the mean of the seven observations in which the ova were kept at 26° at the time of impregnation. In the next three observations in the table, in which the mean time of exposure was 2·5 hours, the mean effect produced was 4·1 per cent. In the 10th hour it was 1·4 per cent., in the 15th to 17th hours on an average 0·36 per cent., and in the 83rd hour on an average only 0·008 per cent. All the observations made, therefore, whether taken in the small groups in which they were

originally obtained, or taken collectively, agree in showing that the effect of temperature on the growth of an organism diminishes very rapidly from the time of impregnation onwards. It is to be noticed that the effect produced was, as far as could be ascertained, a permanent one. At least it persisted to the full larval growth of the organisms, for the larvæ were found to practically cease growing after six to eight days' development. How much would have persisted through the metamorphosis to the adult Echinoid stage is, of course, another matter.

It seems highly probable that what is true for temperature is true for other environmental conditions, and that future research will justify one in assuming the existence of a definite Law of Variation. This might be worded as follows : " *The permanent effect of environment on the growth of a developing organism diminishes regularly and rapidly from the time of impregnation onwards.*"

It is necessary for me now to make one serious criticism of all these observations, one which I regret to say did not occur to me till after they had been completed, and when it was too late for me to put it to a proper experimental test. This criticism depends on the obvious fact that all organisms must be confined within comparatively narrow limits in their powers of growth, so that, for instance, supposing a *Strongylocentrotus* pluteus under average conditions attains a size of 100, then probably under no conditions whatsoever could it be made to attain a larger size than 120 to 125, or a smaller one than 80 to 75. Thus in the most extreme variation noticed in any of the numerous observations made on these larvæ, the range ran from 19·2 per cent. above the normal to 18·0 per cent. below it. Now supposing that during the first hours of development an embryo is placed under especially favourable conditions, then it may happen that thereby it is stimulated to undergo all, or nearly all, the increased growth of which it is capable. In subsequent hours, therefore, little if any more favourable effect may be produced, simply because the organisms from their very nature are unable to show it.

If this principle be examined in relation to the present experiments, I think it can be shown, however, that though no doubt the relation between the reaction of the organism during the earlier hours to that in the later hours has thereby been exaggerated, yet that there still remains plenty of evidence behind to prove that the diminishing reaction to environment exists in addition.

Let us first consider the three series of experiments in which the developing ova were kept at 26° or 25°. Here a diminution of size amounting to from 7·36 to 20·76 per cent. is produced by the first few hours' exposure to high temperature, so that after this, when the environment begins to exert a favourable influence, we know that it has at least this range of growth capacity at its disposal, plus what-

ever amount of increased growth one might have been able to effect in the "normal" larvæ, by exposing them to the most favourable conditions of growth possible. Now we see that in no case did the favourable environment succeed in forcing on the growth of the larvæ to that of the original normal larvæ, so there was always plenty of growth capacity at its disposal.

In the experiment in which the ova were kept at 12° instead of 22·5°, there is no doubt that the larvæ could have been diminished at least 10 to 15 per cent. more if the conditions had only been sufficiently unfavourable and sufficiently long continued. Thus in the above-mentioned paper it is shown* that larvæ kept during the whole period of development at 10°, instead of about 20°, are diminished in size by no less than 24 per cent.

We see, therefore, that in two of the different methods adopted for acting on the larvæ there was always a considerable amount of growth capacity still present. This may have been true also for the third method, though in this case one cannot prove it. Now we have seen in the above tables that the reaction in the latest periods of development was not a hundredth or even a five hundredth part of that in the first hour, and hence, even admitting the growth capacity was diminished, there can be no doubt whatever that the sensitiveness of the organism to the environment undergoes an enormous gradual diminution.

In order to determine exactly the sensitiveness of the developing ova to environment during the various stages of growth, one should keep various portions of them at the normal temperature for the first three, six, &c., hours, and then expose them to the abnormal temperature for a few hours. Then they should be transferred, for the remainder of their developmental period, to the normal temperature. In this way there would always be the same amount of growth capacity for the environment to work upon, and so the effects obtained for the various periods would accurately express the true capacity for reaction.

The Effect of other Environmental Conditions.

It is obvious that in order to demonstrate the principle under discussion, almost any sufficiently powerful condition of environment might have been chosen. Temperature was hit upon first as being the most convenient one, but further series of experiments were made with another condition also, that of salinity of the water. It has been shown† that growth of the larvæ in water of a certain dilution may increase the size by as much as 15·6 per cent., whilst growth in pure

* 'Phil. Trans.,' B, 1898, p. 481.
† 'Phil. Trans.,' B, 1895, p. 587.

sea water, instead of Aquarium tank water, may increase it by as much as 19·2 per cent.* As a rule, however, the effect produced is not so great as this.

The developing ova, after impregnation for one hour under normal conditions in ordinary Aquarium tank water, were kept for various periods in diluted sea water or pure sea water, and were then transferred to ordinary tank water again. Once the ova have reached the free-swimming blastula stage, or within about five hours in the middle of the summer, it is practically impossible to separate them from the water in which they are swimming. In all the experiments, therefore, one part of the diluted or pure sea water, after vigorous stirring to distribute the embryos evenly through it, was poured into ten parts of the normal water. The subsequent growth of the embryos was therefore continued in tank water containing an eleventh part of the foreign water, but, as will soon be seen, this could have made very little difference to their size.

In the first experiment, made in the beginning of April, the developing ova were placed in diluted water made by adding 100 c.c. of fresh water to 1900 c.c. of Aquarium tank water. The specific gravity of this water was found to be 1·02736 at 15·56° C., whilst that of the unadulterated tank water was 1·02869. The following were the results obtained :—

Normal larvæ (13·8°) ...	...	100·00
1—6 hours in diluted water	...	95·44
1—12 „ „	...	93·55
1—25 „ „	...	89·02
1—192 „ „	...	102·08

Here we see that larvæ kept first in diluted water, and then transferred to normal water, are considerably diminished in size, those transferred after twenty-four hours' development being diminished by no less than 11 per cent.

The next experiment was made in July, when the temperature of the water during development was 21·5°, or nearly 8° higher than in the April experiment. The following values were obtained :—

Normal larvæ (21·5°) ...	...	100·00
1—6 hours in diluted water	...	96·90
1—11 „ „	...	96·63
1—25 „ „	...	103·28
1—144 „ „	...	103·66

In this case the larvæ reached their minimum size after ten hours' growth, and then so rapidly increased that fourteen hours later they were 3 per cent. larger than the normal.

* ' Mittheilungen a. d. Zool. Stat. zu Neapel,' vol. 13, p. 376.

The next and last experiment was made in August, when the temperature of the water was on an average 24·5°. In this case the larvæ were first kept in pure sea water collected several kilometres from the shore. The specific gravity of this water was 1·02868 at 15·56°, that of the normal tank water being 1·02901.

Normal larvæ (24·5°)	...	...		100·00
1—4 hours in pure sea water	...			90·38
1—8 ,, ,, ,,	...			93·61
1—12 ,, ,, ,,	...			96·98
1—22 ,, ,, ,,	...			97·04
1—144 ,, ,, ,,	...			103·61

Here we see that the larvæ were reduced to their minimum size by only three hours' development in pure sea water, and that longer treatment produced a more and more favourable effect, though only those larvæ kept for the whole period of development in the pure water were larger than the normal.

We see, therefore, that in each of these series of experiments, though the ultimate effect of the diluted or pure sea water was a favourable one, yet a temporary immersion in it was always unfavourable. The times of production of the maximum diminution of size were respectively twenty-four, ten, and three hours, or apparently very variable. It is to be noticed, however, that these times more or less correspond with the period at which the developing ova reach the free-swimming blastula stage. Thus at a temperature of 13·8° this was found to be some twenty to twenty-four hours, whilst at a temperature of 24° it was about five hours. At 21·5° it is probably about eight hours, though no exact observations were made to determine it.

To what is this unfavourable effect upon the larval growth due? It is impossible that the pure sea water can of itself be a less favourable medium for the early stages of development than the impure tank water, and probably the same is true as regards the diluted tank water. In all probability the harmful effect is to be attributed to the shock attendant on the transference of the embryos from water of a lesser degree of salinity to that of a greater. Owing to the differences of osmotic pressure thereby set up, the tissues would immediately undergo a certain amount of shrinkage, and it is a ready assumption that their growth is thereby for a time delayed. The sensitiveness of the embryos to a change of salinity would seem to be less and less the more advanced the state of development, so that after a day or two's growth the harmful influence becomes entirely in abeyance.

The reverse process of transference of the developing embryos from more saline to less saline water does not, on the contrary, appear to be

attended with any unfavourable result. Thus some of the same stock
of impregnated ova used in the second of the above experiments were
kept for respectively five and twenty-four hours in ordinary tank
water, and were then transferred to diluted water, and kept there for
the remainder of their development. The larvæ so obtained were
respectively 2·6 and 2·8 per cent. larger than the normal, or but
slightly smaller than the larvæ kept for the whole period of develop-
ment in diluted water. In another instance, also, embryos kept for
respectively twenty-three hours and two days in normal water, and for
the rest of development in diluted water, were 3·2 per cent. and 1·1 per
cent. larger than the normal.

These experiments therefore prove that the condition of salinity is
not a favourable one for the determination of the reaction to environ-
ment. Still they serve to emphasise the extraordinary sensitiveness
of the embryos to their environmental conditions, and also show that
this sensitiveness is much greater in the earlier stages of development
than in the later ones. Only one observation was made on the effect
of keeping the ova in diluted water for an hour at the time of impreg-
nation. In this case a diminution of 2·2 per cent. was produced in the
size of the larvæ. In the paper already mentioned, however, five
experiments of this nature are recorded,* the water being of various
degrees of salinity. The effects produced were respectively $-4·3$,
$+4·1$, $-1·8$, $-2·9$, and $-2·4$ per cent., or on an average $-1·5$ per
cent.

Summary.

By keeping the impregnated ova of the Echinoid *Strongylocentrotus
lividus* for various periods during development at an abnormal tem-
perature, and comparing the size of the larvæ into which they developed
with that of larvæ allowed to grow throughout under normal con-
ditions, it was proved that *the permanent effect of temperature on the growth
diminished rapidly and regularly from the time of impregnation onwards.*
For instance, it was found that exposure of the ova to a temperature
of about 8° for an hour at the time of impregnation produced an
average diminution of 4·1 per cent. in the size of the larvæ measured
after eight days' growth; during the 4th hour after impregnation the
diminution produced for each hour's exposure was about 1·2 per cent.,
and during the 15th hour about 0·2 per cent. In another series,
exposure to a temperature of 22° produced an increase in size, this
amounting to about 1·1 per cent. for each hour's exposure in the 4th
hour; to 0·4 per cent. in the 14th hour; 0·13 per cent. in the 46th
hour, and 0·01 per cent. in the 120th hour.

Exposure to a temperature of 26° during the first few hours of
development produced a diminution of from 20·8 to 7·4 per cent.,

* ' Phil. Trans.,' B, 1895, p. 588.

but in the later hours it produced an increase of from 4·3 to 11·0 per cent. The reaction of the organism to a constant environmental condition was thus a variable one. This is probably explicable by the fact that the temperatures necessary to kill the organisms, and presumably also those which cause an unfavourable effect on growth, rise steadily during development. Thus the death temperature is about 28·5° for unsegmented ova, 34° for blastulæ, and 40° for plutei.

The impregnated ova were also found to be much more sensitive to changes in the salinity of the water during the early stages of development than during the later ones.

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" On the Diffusion of Gold in Solid Lead at the Ordinary Temperature." By Sir W. ROBERTS-AUSTEN, K.C.B., F.R.S., Professor of Metallurgy, Royal College of Science. Received April 5,— Read May 10, 1900.

In the Bakerian Lecture, " On the Diffusion of Metals,"* delivered in 1896, evidence was given to show that gold placed at the base of a column of fluid lead 16 cm. high, maintained at a mean temperature of 492°, or 166° above the melting point of lead, diffuses to the top of the column in an appreciable amount in a single day, the diffusivity expressed in centimetre-day units being 3·0. If the lead be heated, say to 251°, or 75° below the melting point of the metal, diffusion takes place at a much slower rate ; it may still be readily measured, though the diffusivity is only 0·023 in centimetre-day units. In the experiments on diffusion in solid lead, the latter metal was prepared with great care, and possessed a high degree of purity. The method of preparation consisted in the reduction of carefully purified carbonate of lead by cyanide of potassium, the reduced metal being cast in carbon moulds.

It became evident that at the ordinary temperature the rate of diffusion of solid gold in solid lead must be very slow, and I stated in the Bakerian Lecture that cylinders of lead had been set aside with discs of gold affixed to their bases, in order that, after a sufficient lapse of time, the diffusion occurring at the ordinary temperature might be measured. By the month of March in the present year, four years had elapsed since the experiment began, and the time appeared to be sufficiently long to justify the attempt to ascertain how far the gold had diffused. In starting the experiments the bases of the lead cylinders were carefully brought to a smooth surface, and the discs of pure gold were specially cleaned, the discs of gold being held against

* Delivered February 20, 1896. ' Phil. Trans.,' A, vol. 187 (1896) pp. 383—415.